Thesis

Comparing atrial strain between Hypertrophic Cardiomyopathy and Cardiac Amyloidosis

submitted by

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In partial fulfilment of the requirements for the degree of

Doktorin der gesamten Heilkunde

(Dr^{in}. med. univ.)

at the

Medical University of Graz

Executed at the

Department of Internal Medicine

Division of Cardiology

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Graz, 11.12.2024

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Graz, 11.12.2024 *Larissa Sarah Winkelbauer m.p.*

Acknowledgements

First, I am thankful for the opportunity to join the Research Team of Nicolas Verheyen, where I learned a lot and was also provided with useful tips and plenty of inspiration along the way. Second, I want to thank David Zach, my supervisor, for always providing guidance and showing patience.

And without the never-ending support and encouragement of my partner and my parents, many things would not have been possible. I want to thank them and my friends for always being there for me.

Zusammenfassung

Die Zulassung von neuen mortalitätsreduzierenden Medikamenten für die kardiale Amyloidose hat zu einer vermehrten Überweisung dieses PatientInnen-Kollektivs in tertiäre Zentren geführt. Neue diagnostische Zugangswege sind von hoher Bedeutung, da eine frühe Diagnosestellung hohe Relevanz für den Krankheitsverlauf sowie die Mortalität hat. Daher betrachtet diese Studie die diagnostische Akkuratheit von Links- und Rechts atrialen Reservoir Strain (LASr und RASr) bei der Differenzierung zwischen kardialer Amyloidose und hypertropher Kardiomyopathie.

Hier wurde eine Querschnittsanalyse mithilfe des Graz HCM Registers durchgeführt. Das Graz HCM Register ist eine prospektiv geführte single-center Studie, welche PatientInnen mit einem hypertrophen Phänotyp in der Echokardiographie einschließt. Jeder Patient/jede Patientin in dieser Studie hat eine standardisierte transthorakale Echokardiographie erhalten. Die post-processing Strainanalyse wurde von verblindeten UntersucherInnen durchgeführt. In diese Studie wurden 100 PatientInnen mit HCM und 95 PatientInnen mit CA eingeschlossen.

Verglichen mit HCM-PatientInnen waren CA-PatientInnen häufiger männlich [79 (83%) vs. 54 (54%); p<0.001] und älter [78 (73-81) vs. 58 (47-70) years; p<0.001]. Zusätzlich wiesen CA-PatientInnen einen niedrigeren BMI [25.0 (22.8-26.9) vs. 26.5 (24.3-29.7) kg/m²; p=0.002] sowie niedrigeren systolischen [134±20 vs. 142±25 mmHg; p=0.018] und diastolischen Blutdruck [78±12 vs. 84±12 mmHg; p<0.001] auf. Weiters war das NTproBNP [2533.0 (1146.0-5016.0) vs. 543.0 (195.0-1381.0) pg/ml; p<0.001] deutlich höher und die eGFR [58.4±19.1 vs. 80.1±22.2 ml/min/1.73m²; p<0.001] deutlich niedriger bei CA-PatientInnen im Vergleich zu HCM-PatientInnen. Der LASr und RASr war signifikant niedriger in CA-PatientInnen als in HCM-PatientInnen [9.2 (5.6-13.0) vs. 21.7 (14.8-27.6) %; p<0.001] [11.2% (16.4) vs 26.7±11.9%; p<0.001]. In einer multivariablen logistischen Regressionsanalyse zeigte sich LASr als unabhängiger Prädiktor für kardiale Amyloidose, adjustiert für Kovariablen [OR=0.936 (95% CI 0.877-0.998)]. Eine ROC-Analyse zeigte eine AUC von 0.8 (95% CI 0.73-0.86) für LASr und ebenso 0.8 (95% CI 0.72-0.86) für RASr. Ein optimaler Grenzwert von ≤ 14.3 % bei LASr und ≤ 23.7% bei RASr zeigte eine hohe Sensitivität und Spezifität zur Differenzierung beider Erkrankungen [LASr 80%, Sensitivität 79% Spezifität; RASr 68% Sensitivität, 84% Spezifität]. Beide Marker waren ebenso mit der diastolischen Funktion als auch kardialen Biomarkern

(NTproBNP und hs-TnT) assoziiert. Eine Sensitivitätsanalyse mit 74 Alters- und Geschlechts-angepassten Patienten zeigte unveränderte Ergebnisse.

Im Vergleich zu HCM-PatientInnen, war LASr und RASr signifikant niedriger bei CA-PatientInnen. LASr war ein unabhängiger Prädiktor für kardiale Amyloidose. LASr und RASr wiesen eine hohe diagnostische Akkuratheit auf.

Abstract

Aims: Availability of mortality-reducing therapies for cardiac amyloidosis (CA) has led to an increase in referral rates to tertiary care centers. Investigations to improve diagnosis rates are urgently needed, as diagnosis of CA requires a multimodal approach, and its early diagnosis is crucial due to high mortality in untreated patients. The aim of this study is to evaluate the accuracy of the left and right atrial reservoir strain (RASr & LASr) in differentiating CA patients from patients with hypertrophic cardiomyopathy (HCM).

Methods: This is a cross-sectional analysis from the Graz HCM Registry, a prospective single-center cohort study enrolling consecutive patients with a HCM phenotype. This analysis included HCM and CA patients with valid standardized transthoracic echocardiographic examinations. Blinded investigators performed post-processing echocardiographic analyses in 100 HCM and 95 CA patients.

Results: Compared to HCM patients, CA patients were more frequently male [79 (83%) vs. 54 (54%); p<0.001] and older [78 (73-81) vs. 58 (47-70) years; p<0.001]. Moreover, CA patients had a lower BMI [25.0 (22.8-26.9) vs. 26.5 (24.3-29.7) kg/m²; p=0.002] as well as lower systolic [134±20 vs. 142±25 mmHg; p=0.018] and diastolic blood pressure [78±12 vs. 84±12 mmHg; p<0.001]. Additionally, CA patients had higher NT-proBNP [2533.0 (1146.0-5016.0) vs. 543.0 (195.0-1381.0) pg/ml; p<0.001] and lower eGFR [58.4±19.1 vs. 80.1±22.2 ml/min/1.73m²; p<0.001]. LASr and RASr was significantly worse in CA patients compared to HCM patients [9.2 (5.6-13.0) vs. 21.7 (14.8-27.6) %; p<0.001] [11.2% (16.4) vs 26.7±11.9%; p<0.001]. Multivariable logistic regression analysis revealed LASr as an independent predictor of CA [OR=0.936 (95% CI 0.877-0.998)], adjusting for covariates. ROC analysis showed an AUC of 0.80 (95% CI 0.73-0.86) for LASr and 0.80 (95% CI 0.72-0.86) for RASr to discriminate CA from HCM. LASr ≤14.3% demonstrated an 80% sensitivity and 79% specificity for the identification of CA patients. RASr ≤ 23.7% showed a 68% sensitivity and 84% specificity. Both markers are associated with diastolic dysfunction and cardiac biomarkers (NTproBNP and high sensitivity Troponin). Sensitivity analyses involving 74 sex- and age-matched patients yielded materially unchanged results.

Conclusion: Compared to HCM patients, LASr was diminished in CA patients and an independent predictor of CA. Moreover, LASr and RASr showed high diagnostic accuracy in differentiating CA and HCM patients.

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List of Abbreviations

ACE	Angiotensin-converting enzyme
AL	Immunoglobulin Light chain Amyloidosis
ASE/EACVI	American Society of Echocardiography/European Association of Cardiovascular Imaging
AT1	Angiotensine II receptor type 1
ATTR (ATTRwt, ATTRv)	Transthyretin Amyloidosis (wildtype, variants)
AUC	Area under Curve
BMI	Body mass index
CA	Cardiac Amyloidosis
CI	Confidence interval
CKD-EPI	Chronic Kidney Disease – Epidemiology Collaboration
CMR	Cardiac magnetic resonance
CW	Continuous wave
DBP	Diastolic Blood Pressure
DPD	3,3-Diphosphono-1,2-Propanodicarboxylic acid
DT	Deceleration time
ECG	Electrocardiogram
EDV	End diastolic Volume
EF	Ejection fraction
eGFR	Estimated glomerular filtration rate
ESC	European society of Cardiology
ESV	End systolic Volume
FLC (dFLC)	Free light chain (Difference between involved and uninvolved FLC)
GLS	Global longitudinal strain
HCM	Hypertrophic Cardiomyopathy
HF	Heart failure
HFpEF	Heart failure with preserved Ejection Fraction
HMDP	Hydroxymethylene-Diphosphonate
Hs-cTnT	Highly sensitive cardiac Troponin T
ICD	Implantable cardioverter-defibrillator
IVRT	Isovolumetric relaxation time
IVS	Interventricular septum

LA	Left atrium
LASr	Left atrial reservoir strain
LAVi	Left atrium volume index
LGE	Late gadolinium enhancement
LV	Left ventricle
LVH	Left ventricular hypertrophy
LVOT(O)	Left ventricle outflow tract (obstruction)
MR	Mitral regurgitation
MV	Mitral valve
NT-proBNP	N-terminal pro-brain natriuretic peptide
NYHA	New York Heart Association
OR	Odds ratio
PET	Positron emission tomography
PV	Pulmonary vein
PWT	Inferolateral/posterior wall thickness
PYP	Pyrophosphate
RA	Right atrium
RASr	Right atrial reservoir strain
RELAPS	Relative apical sparing
ROC	Receiver Operating Characteristic
ROI	Region of Interest
RUPV	Right upper pulmonary vein
RV	Right ventricle
SBP	Systolic Blood Pressure
SGLT2	Sodium-Glucose-cotransporter 2
SPIE	Serum protein electrophoresis with immunofixation
SR	Sinus rhythm
SRT	Septal reduction therapy
TAVR	Transfemoral aortic valve replacement
TDI	Tissue derived imaging
TR	Tricuspid regurgitation
UPIE	Urine protein electrophoresis with immunofixation

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1 Introduction

1.1 Introduction to Cardiac Amyloidosis

Cardiac Amyloidosis is emerging as a disease with significant impact for patients and clinicians. It can now be diagnosed without invasive biopsy and is treatable when detected early. A transthyretin tetramer stabilizer (Tafamidis) is available and demonstrates promising results regarding reduction of cardiovascular-related hospitalizations, all-cause mortality and quality of life. (1) Due to the availability of viable treatment, more referrals to tertiary centers are noted, therefore it is an additional burden for hospitals and clinicians.

A common misconception is that CA is rare, but an autopsy study found that 25% of people over the age of 80 to 85 had ATTR amyloidosis.(2) Furthermore, 13% of 120 patients with HFpEF and 16% of 151 patients undergoing TAVR for severe calcific aortic stenosis were found to have ATTR-CA. (3, 4)

Cardiac Amyloidosis is still associated with a high mortality rate. The median survival from diagnosis is 24 months in AL-CA, 57 months in ATTRwt-CA and 31 to 69 months in ATTRv-CA, depending on the mutation.(5)

1.1.1 Epidemiology of Cardiac Amyloidosis

Approximately a fourth of patients over 80-85 years of age has ATTR-Amyloidosis, although the exact prevalence is unknown. Wild-type ATTR Amyloidosis typically affects older patients, with diagnoses often occurring between the 7th to 10th decade. In contrast, variant ATTR-CA patients are diagnosed at a younger age, between the 3rd to 8th decades. AL-CA is rarer than ATTR-CA and is typically diagnosed earlier, generally between the 5th to 9th decades. While AL-CA affects both genders, it is slightly more common in men. Conversely, ATTR-CA shows a strong male predominance.(6)

1.1.2 Pathophysiology of Cardiac Amyloidosis

This disease involves an infiltrative process, where amyloid fibrils accumulate in the extracellular space between cardiac myocytes. This results in a non-compliant and stiff heart, which loses its ability to distend, affecting all four cardiac chambers.(7)

Various forms of systemic amyloidosis have different phenotypes and prognoses. Over 98 % of currently diagnosed cardiac amyloidosis cases are due to AL or ATTR, either hereditary (ATTRv) or acquired (ATTRwt). Rarer forms are secondary to chronic inflammatory and infectious diseases (AA).

In AL Amyloidosis, the fibrils are formed of monoclonal immunoglobin light chains. This condition frequently occurs with clonal plasma cell or other b-cell dyscrasias. While various organs can be affected, cardiac involvement is present in 50% of cases.

Hereditary ATTR amyloidosis is caused by mutations in multiple genes, with the transthyretin gene being the most common. These mutations reduce the stability of the tetrameric structure of transthyretin, a transporter protein for thyroid hormone and retinol. Over 120 mutation variants are known, some of which correlate with specific geographic regions or ethnic groups. This disorder affects men and women equally and primarily presents progressive peripheral and autonomic neuropathy combined with cardiac involvement, although primarily cardiac phenotypes are possible.

In Senile Systemic Amyloidosis or Wild-Type-ATTR-Amyloidosis, the dominant feature is cardiac involvement. Extracardiac involvement is rare. Wild-type TTR amyloidosis deposits are frequently found in autopsies of individuals over 80 years of age and exhibit a strong male predominance. Beyond this, little is known about the pathophysiological process. (5-7)

1.1.3 Comparison with Hypertrophic Cardiomyopathy

Hypertrophic cardiomyopathy is histologically described as a disorganised arrangement of hypertrophic myocytes with interstitial fibrosis. (8)
Numerous factors, like elevated LV pressure, pathological ventricular contraction and relaxation as well as prolonged activation of intracellular calcium channels, contribute to the diastolic dysfunction, seen in HCM patients. Myocardial hypertrophy, ischemia, and replacement or interstitial fibrosis can cause chamber stiffness, therefore leading to impairment of ventricular myocardial relaxation. Furthermore, this leads to greater dependency on the atrial stroke volume for ventricular filling.(9, 10)

Both, CA and HCM, have a hypertrophic phenotype, but only in HCM patients' true histological hypertrophy can be found. Where the cells grow bigger, compared to CA, where the extracellular area grows. This can be explained by the different pathophysiology. In HCM, a disorganized arrangement of hypertrophic myocytes with interstitial fibrosis is described. In CA, the extracellular infiltration of amyloid is present. Both diseases suffer from diastolic dysfunction and are part of the HFpEF spectrum. Furthermore, Cardiac Amyloidosis is a disease of the older patients (typically above 60 years of age), in contrast to HCM. (11, 12)

1.2 Importance of Early diagnosis

In ATTR CA, disease specific therapy is available, which reduces amyloidogenesis, e.g. by stabilizing the transthyretin tetramer and achieves the best efficacy when administered in early disease stages. When the disease has not progressed to NYHA class III, disease specific therapy significantly reduces cardiovascular-related hospitalizations and all-cause mortality. However, in patients with NYHA class III, the rate of cardiovascular-related hospitalizations are higher in the tafamidis cohort compared to the placebo cohort. This underlines the importance of early treatment. (1)

1.3 Overview of Existing Diagnostic Methods

There are three different settings, where a diagnostic work-up is indicated. First, patients with unexplained left ventricular hypertrophy, with or without heart failure symptoms or history of syncope and patients with heart failure in combination with red flags for Amyloidosis, such as bilateral carpal tunnel syndrome, peripheral neuropathy, macroglossia, skin bruises or renal dysfunction, should be further evaluated. The same goes for patients with low-flow, low gradient aortic stenosis, myocardial granular sparkling or abnormal left ventricular global longitudinal changes (especially with apical sparing). Also, patients with typical systemic condition like plasma cell dyscrasia, nephrotic syndrome, peripheral neuropathy or a chronic systemic inflammatory condition are at a higher risk for suffering from amyloidosis.(5, 13)

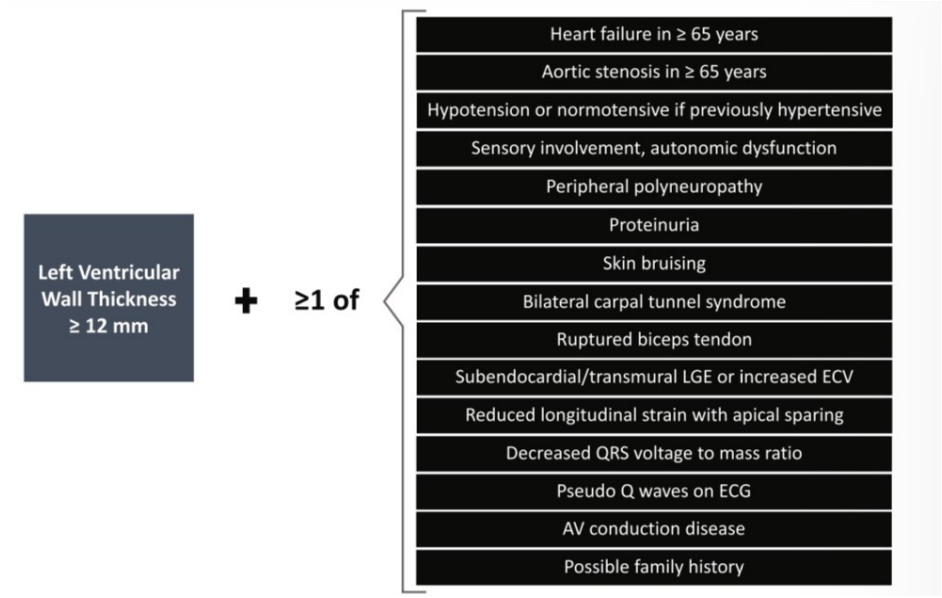


Figure 1: algorithm for further diagnostic work-up, when LV hypertrophy and at least one red flag is present

1.3.1 Current Diagnostic Approaches

Initially, a thorough clinical examination and history needs to be taken, to identify cardiac and extracardiac symptoms, which could be related to Amyloidosis. Typical cardiac work-up (like ECG, echocardiography, cardiac biomarkers) can raise further suspicion. Typical findings are summarized in table 1. Elevation of NT-proBNP values, which are in disproportion to HF symptoms and low-level Troponin T elevation without explanation, are common findings in blood samples.(5, 13)

Table 1: typical findings in cardiac diagnostic work-up

Diagnostic test	**Main findings**
Clinical examination	- Heart failure symptoms with prominent right heart failure (meaning peripheral congestion)
Electrocardiography	- Low-voltage QRS (disproportional to LV wall thickness) - Poor R-wave progression (pseudoinfarction pattern) - Pathological q-waves - Atrial fibrillation - AV conduction abnormalities - Bundle branch block - QT prolongation

Echocardiography	- Unexplained LV hypertrophy - Atrial dilatation - RV free wall hypertrophy - Myocardial granular sparkling (in non-harmonic imaging) or speckle appearance (in harmonic imaging) - Thickening of AV valves and atrial septum - Diastolic LV dysfunction - Abnormal LV GLS with apical sparin (“cherry-on-top” pattern) - Low-flow, low-gradient aortic stenosis
Biomarkers	- Unexplained persistent low-level cTn elevation - Significant NP elevation (disproportional to HF symptoms)
CMR	- Diffuse subendocardial or transmural LGE - High extracellular volume

The ESC position paper recommends a diagnostic algorithm, which includes ^{99m}Tc-PYP, -DPD or -HMDP scintigraphy and screening for monoclonal proteins by serum free light chain (FLC) assay, serum (SPIE) and urine (UPIE) protein electrophoresis with immunofixation. If the myocardial radiotracer uptake is a grade 2 or 3 and monoclonal gammopathy is not present, a diagnosis of ATTR CA can be made. If it is only grade 1, histological evidence of amyloid deposits is needed.

If one or more of the monoclonal protein tests are abnormal and Perugini Score is zero, a CMR should be performed. Histological confirmation of clinically affected organ is recommended to diagnose AL amyloidosis. Furthermore, if Perugini Score is 1 to 3 and the haematologic tests are positive, a biopsy is needed to characterize the subtype of CA. (5, 7)

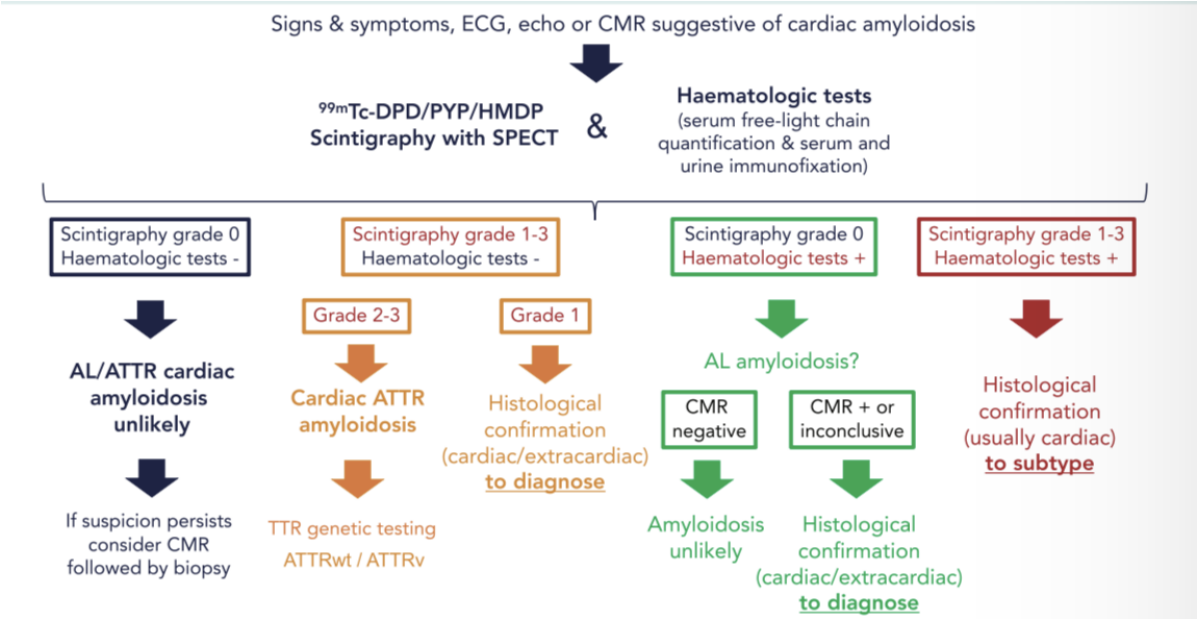


Figure 2: diagnostic algorithm for diagnosing ATTR/AL Amyloidosis recommended by ESC heart failure position paper

1.3.2 Challenges in Diagnosis

Current approaches for suspecting CA require only LVH and one or more of the afore-mentioned red flags. However, the diagnostic work up involves expensive and extensive tests. Often, the results are ambiguous, necessitating further investigation with CMR or a biopsy. Consequently, patients face longer waiting periods and delays in confirming the diagnosis.

1.4 Significance of Atrial Reservoir Strain

Atrial strain represents atrial function and is affected by heightened afterload as in higher LV filling pressures but also intrinsic LA dysfunction. It can be subdivided into reservoir, conduit and contraction strain. Reservoir strain is the only phase, which can be applied to patients with and without atrial fibrillation.

The passive filling of atrial chambers is dependent on the downward motion of the atrioventricular plane and the ventricle filling is dependent on a distendable myocardium to optimise blood flow. Both mechanisms being compromised in hypertrophic cardiomyopathy and its phenocopies.(14)

1.5 Aims of the Study

This analysis focuses on atrial strain in two disease cohorts characterized by LVH and diastolic dysfunction, i.e. hypertrophic cardiomyopathy and cardiac amyloidosis. This study aims to determine 1) differences in atrial function by comparing left and right atrial reservoir strain between HCM and CA patients and 2) the diagnostic accuracy of left and right atrial reservoir strain in identifying CA patients. Moreover, sensitivity analyses were performed in a sex- and age-matched case-control study.

2 Methods

2.1 Study Design

This is a cross-sectional analysis including 100 HCM patients and 96 CA patients from the Graz HCM Registry, a prospective single-center cohort study enrolling consecutive patients with non-adaptive left ventricular hypertrophy since 2019. Non-adaptive left ventricular hypertrophy comprises hypertrophic cardiomyopathy and its phenocopies including infiltrative diseases such as cardiac amyloidosis. Sensitivity analyses were performed in a case-control study comparing 37 adult CA patients with 37 sex- and age-matched HCM patients participating in the Graz HCM registry. Matching was performed in a 1:1 fashion with an age tolerance of 2 years. This study was approved by the institutional review board of the Medical University of Graz (EC-No. 30-286 ex 17/18) and written informed consent was obtained from each participant. This study complied with standards of Good Clinical Practice and the Declaration of Helsinki.

2.2 Study Population

2.2.2 Inclusion criteria

Inclusion criteria were 1) participation in the Graz HCM Registry, 2) age over 18 years, 3) non-adaptive LV hypertrophy due to HCM or CA, and 4) a comprehensive resting transthoracic echocardiographic examination including an apical four chamber view.

2.2.3 Exclusion criteria

Patients with concomitant severe valve diseases were excluded from this analysis, as they are a major confounding factor for atrial strain. (15, 16)

2.2.4 Definition of HCM

HCM was defined according to guidelines from the European Society of Cardiology, American Heart Association, and American College of Cardiology, either as maximal

end-diastolic (ED) LV wall thickness ≥15 mm in one or more left ventricular myocardial segments not explained by loading conditions, or as ED LV wall thickness ≥13 mm in case of familiar HCM or a confirmed disease-causing mutation.(12, 17)

2.2.5 Definition of CA

Cardiac Amyloidosis is a restrictive cardiomyopathy characterized by extracellular accumulation of amyloid fibrils. In Echocardiocraphy, Cardiac Amyloidosis presents as biventricular wall thickness and ventricular stiffness. (18) Cardiac Amyloidosis is a conglumerate of heart failure, syncope, or bradyarrhythmia with typical ECG or CMR findings. Depending on the results of bone scintigraphy, serum-, urine immunofixation and serum free light chain assay, a diagnosis of AL or ATTR Amyloidosis can be made.(19)

2.3 Clinical Characteristics

The following characteristics of the study population were gathered: supine blood pressure, heart failure medication (beta blocker, mineralcorticoid receptor antagonist, ACE inhibitor, AT1 antagonist, diruetics, SGLT2 inhibitors), NYHA class, heart rhythm, presence of permanent pacemaker, genotype of both diseases and septal reduction therapy.
The BMI was calculated by dividing the weight in kilograms by the square of height in meter. Patients with a BMI above 30 kg/m² were classified as obese. The blood pressure was evaluated in a supine position with an oscillometric automatic sphygmomanometer. The New York Heart association (NYHA) functional class is a system to describe the functional level of a patient. Depend on the time when the patient feels short of breath and how much physical exercise he or she can do, classes 1 to 4 are available. The definition of each class can be found in table 2.(20)
A conventional 12-lead electrocardiogram was recorded, as part of the baseline characterization at registry inclusion. The presence of atrial fibrillation, AV-block or bundle branch block was noted. Furthermore, the presence of a pacemaker or implantable cardioverter-defibrillator (ICD) has been assessed.

Table 2. Stages of Heart Failure

Class	Patient symptoms
I	No limitation of physical activity. Ordinary physical activity does not cause undue fatigue, palpitation or shortness of breath.
II	Slight limitation of physical activity. Comfortable at rest. Ordinary physical activity results in fatigue, palpitation, shortness of breath or chest pain.
III	Marked limitation of physical activity. Comfortable at rest. Less than ordinary activity causes fatigue, palpitation, shortness of breath or chest pain.
IV	Symptoms of heart failure at rest. Any physical activity causes further discomfort.

2.4 Bone Scintigraphy

As described before, a ^{99m}Tc-DPD, ^{99m}TC-PYP or ^{99m}Tc-HMDP scintigraphy is part of the diagnostic algorithm for the diagnosis of CA, specifically ATTR-Amyloidosis. For the assessment of the scintigraphy the semi-quantitative Perugini grading scale is used. Grade 0 is noted, when no cardiac uptake and normal rib uptake is present. Grade 1 describes a cardiac uptake which is less than the rib uptake. Grade 2 reports a similar cardiac uptake as rib uptake. And finally Grade 3 is when the cardiac uptake is greater than rib uptake, while there is mild or absent rib uptake. Grade 2 is the cut-off value, therefore Grade 2 or more are classified as ATTR positive, when light chain-assessment is obtained.(21)

2.5 Staging

As a way of staging ATTR Amyloidosis patients the UK NAC ATTR staging score is used. This defines 3 stages, by defining different levels of NT-proBNP and eGFR. The different stages are visualized in the table below.(22)

Stage	*NT-proBNP > 3000 ng/L*	*eGFR <45 ml/min/1.73m²*
Stage 1	No parameter outside of normal range	
Stage 2	One parameter outside of normal range	
Stage 3	Both parameters outside of normal range	

The Mayo Clinic Staging System for AL amyloidosis was updated in 2012 and establishes three risk factors. Depending on the quantity of elevated risks, Stages 1 to 4 are defined. The risk factors include dFLC (cutoff of 18 mg/dl), NT-proBNP (cutoff of 1800 ng/L) and Troponin T (cutoff of 0.025 mcg/L). Stage I is present, if no risk factor is above the cutoff value, and depending on the quantity of elevated risk factors, stage 2 to 4 is present.(23)

Stage	*dFLC > 18 mg/dl*	*NT-proBNP >1800 ng/L*	*Troponin T > 0.025 mcg/L*
Stage 1	None is above the cut off		
Stage 2	One is above the cut off value		
Stage 3	Two are above the cut off value		
Stage 4	All are above the cut off value		

2.4 Laboratory Indices

The plasma-derived cardiac biomarkers NT-proBNP and hsTnT were used. NT-proBNP as well as hsTnT were analysed using an electrochemiluminescence immunoassay (Roche Diagnostics).
Renal parameters as Creatinine and eGFR were also included. Creatinine was determined using photometry (Roche Diagnostics). eGFR was calculated by using the CKD-EPI equation.(24)

2.5 Echocardiographic Analysis

2.5.1 Acquisition

A comprehensive resting transthoracal echocardiography with ECG gating was performed at the time of inclusion in the Graz HCM registry. The images and loops were stored in the application “IntelliSpace Cardiovascular” by Philips.

2.5.2 Analysis

For each patient, the quality of echocardiographic images was analyzed. Loops with the best quality showing the total circumference of the atrial chambers were selected. Atrial strain was measured offline, blinded to participants’ clinical characteristics, using a dedicated vendor-independent post-processing software (TomTEC Arena including 2D cardiac performance Analysis, TomTEC Imaging Systems).

2.5.3 Chamber quantification

There are two established ways to determine the LV wall thickness, this is specifically important for the diagnosis of HCM. The most common way is via echocardiography, here either M-mode tracing or in 2D a linear internal method can be used. In the long or short parasternal view, the wall thickness is measured at end-diastole in a 90-degree angle to the LV long axis. The interventricular septum (IVS) and the inferolateral wall thickness (PWT) are the obtained measurements. (25)

Table 3: Normal ranges of chamber quantification

Linear method	*Women*	*Men*
Septal thickness [cm]	0.6-0.9	0.6-1.0
Posterior wall thickness [cm]	0.6-0.9	0.6-1.0
Relative wall thickness [cm]	0.22-0.42	0.24-0.42

2.5.4 LVOTO

LVOTO was defined as a peak LVOT gradient ≥ 30 mmHg determined by CW Doppler.(11, 26)

2.5.4 Systolic Dysfunction

Ejection fraction indicates the amount of blood ejected during each cardiac cycle and is a surrogate for LV systolic function. It is calculated using the following formula EF = (EDV-ESV)/EDV. According to the ASE/EACVI guidelines, a biplane method of disks is the currently recommended assessment of EF.(25)

2.5.5 Diastolic Dysfunction

The ASE/EACVI Guidelines proposed an algorithm to grade diastolic dysfunction. Diastolic function is per definition not normal in patients with cardiomyopathies. The scheme is used to grade diastolic function in indeterminate, elevated or non-elevated LV filling pressure by assessing Average E/e', septal e' velocity, lateral e' velocity, TR-velocity and LA volume index. Furthermore, by investigating the mitral inflow, grading from Grade 1 to Grade 3 can be achieved. Hereby E/A ratio and E velocity is used.(10)

Diastolic Dysfunction in HCM

To quantify diastolic dysfunction in patients with HCM, the ASE/EACVI Guidelines recommend using following echocardiographic parameters: average E/e', LA volume index, pulmonary vein atrial reversible velocity (S wave, D wave, S/D ratio, AR duration) and peak velocity TR jet by CW-Doppler. These parameters can be used irrespective of the presence or absence of dynamic obstruction or mitral regurgitation. Only if MR is present in moderate severity, only peak velocity of TR and Ar-A duration is valid.(9, 10)

Diastolic Dysfunction in Amyloidosis

Cardiac Amyloidosis is part of the restrictive cardiomyopathy group, therefore there are different markers, which can be applied to quantify diastolic function in individuals. To stage the degree of diastolic dysfunction, parameters like mitral inflow E/A ratio, DT of E velocity, IVRT and septal and lateral e' velocities should be measured. (10)

Current recommendations of diastolic function quantification

Peak E-wave velocity (cm/sec) during mitral inflow is measured at early diastole, i.e. after the T wave on ECG, using PW-Doppler as a surrogate of the LA-LV pressure gradient. It represents blood flow into the LV at early diastole, which occurs due to LV relaxation.
Peak A-wave velocity (cm/sec) displays the mitral inflow at late diastole (after ECG P wave) and reflects the LA-LV pressure gradient and is influenced by LV compliance and atrial contraction.
Mitral valve E/A ratio can be used to determine LV filling patterns and differentiate in normal, impaired relaxation, pseudonormal and restrictive filling. Mitral inflow parameters are limited in their use in patients with arrhythmias, especially atrial fibrillation or atrial flatter and they are all age dependent. Additional limitations are the presence of significant mitral valve disease, LV assist devices, left bundle branch block and ventricular paced rhythm.
MV DT (msec) measures a time interval between peak-E wave and zero-velocity baseline by following and extrapolating the slope of LV filling. The use of MV DT is only recommended in patients with reduced EF.
Pulsed-wave TDI e' velocity (cm/sec) describes tissue doppler which is located at the septal or lateral mitral anulus and measures the LV relaxation at early diastole. It is a marker for impaired LV relaxation, and it is relatively independent of LV filling pressures.
PV S wave and D wave (cm/sec) measure the peak velocity, either at early systole or early diastole, in the right (or left) upper pulmonary vein. S-wave has various influences, namely LA pressure changes, LA, LV and RV contractility. Early LV diastolic LV filling influences D-wave.
CW Doppler TR systolic jet velocity (m/sec) is assessed by using continuous wave doppler on tricuspid regurgitation. If a pulmonary parchenymal or vascular disease can be excluded, this marker correlates with LA pressure. (10)

2.5.6 Speckle tracking echocardiography

Speckle Echocardiography traces the natural speckle pattern of the myocardium during the contraction and measures the covered distance during the cardiac cycle. Therefore, the acquired parameter is the global longitudinal strain, which is defined as the percentage of fibre length during maximum contraction at end-systole compared to the length in a relaxed state at end-diastole. (27)

According to the consensus paper of the European Society of Cardiology, the recommended views are the apical four-chamber and two-chamber view, in this analysis, apical four-chamber view was used to measure atrial strain.
First the region of interest (ROI) was defined, by tracing the endocardial border beginning with the mitral anulus, extrapolating across the pulmonary veins and/or left atrium appendage orifices, all the way to the opposite mitral annulus side. As the atrial wall is thin to begin with, it does not matter whether the endocardial and the epicardial border are defined or the software uses a default width of 3 mm. Before measuring the left atrial cardiac cycle is defined. It is subdivided in three phases:

- Reservoir phase: This phase starts with the mitral valve closure, which marks the end of ventricular diastole and goes on until the same valve opens.
- Conduit phase: This phase marks the time between mitral valve opening and atrial contraction. In the presence of atrial fibrillation there is no designated atrial contraction, and this phase continues until the mitral valve closes again.
- Contraction phase: This phase is defined as the time of atrial contraction till the mitral valve closes.

There are two possible options for the zero reference, the end-diastole or onset of atrial contraction. The use of onset of atrial contraction as zero reference is only indicated in patients with sinus rhythm, whereas end-diastole can be used on patients with atrial fibrillation/flutter as well. In this analysis, end-diastole was used as the reference point, as atrial fibrillation was frequently present.

The same applies to the right atrial strain measurement. The apical four-chamber-view is recommended, and the region of interest is defined by the endocardial border beginning at the tricuspid valve anulus, following the right atrium side wall, roof, atrial septum to the opposite tricuspid anulus side. (28)

2.6 Statistical Analysis

The data was analyzed using the SPSS statistical software package (SPSS Inc., IBM Company). A two-tailed p-value < 0.05 was considered significant. Categorical variables are expressed as counts (percentage), and continuous normally distributed variables as mean ± standard deviation and continuous non-normally distributed variables as median (interquartile range). Normal distribution was assessed using the Shapiro-Wilk-test and QQ-plots. For group comparison of characteristics between CA and HCM, Fisher's exact, chi-squared, Mann-Whitney U or independent samples t-test was performed. A ROC Analysis was performed, and the Youden Index was calculated to gather the ideal cut off value with corresponding sensitivity and specificity. To validate the findings, a sensitivity analysis was conducted in an age- and sex-matched cohort.

3 Results

3.1 Baseline Characteristics – Total cohort

TABLE 1: BASELINE CHARACTERISTICS.

	Available in N (total/CA/HCM) (%)	Total Cohort N=195	CA N=95	HCM N=100	P-Value
BASELINE CHARACTERISTICS					
AGE, YEARS	195 (100)	71 [56-79]	78 [73-81]	57.1±16.2	**<0.001**
FEMALE, N (%)	195 (100)	62 (31.8)	16 (16.8)	46 (46)	**<0.001**
SBP, MMHG	169 / 82 / 87	136.0 [123.0-155.5]	133.6 ± 20.2	140 [126-158]	**0.006**
DBP, MMHG	169 / 82 / 87	81.2 ± 12.4	78.0 ± 12.4	84.3 ± 11.7	**<0.001**
BMI, KG/M²	180 / 87 / 93	25.7 [23.7-28.1]	25.0 [22.8-26.9]	26.5 [24.3-29.7]	**0.002**
NYHA CLASS I, N (%)	180 / 86 / 94	41 (22.8)	13 (15.1)	28 (29.8)	
NYHA CLASS II, N (%)		57 (31.7)	21 (24.4)	36 (38.3)	
NYHA CLASS II-III, N (%)		30 (16.7)	19 (22.1)	11 (11.7)	
NYHA CLASS III, N (%)		47 (26.1)	30 (34.9)	17 (18.1)	
NYHA CLASS IV, N (%)		5 (2.8)	3 (3.5)	2 (2.1)	
LABORATORY INDICES					
PROTEIN CREATININE RATIO, MG/G	175 / 85 / 90	111.0 [71.0-185.0]	125 [89.5-218]	90 [63.8-146.5]	**<0.001**
EGFR, ML/MIN/1.73M²	190 / 91 / 99	69.7 ± 23.4	58.4 ± 19.1	80.1 ± 22.2	**<0.001**
NTPROBNP, NG/ML	190 / 91 / 99	1150.0 [352.0-3032.5]	2533.0 [1146.0-5016.0]	543.0 [195.0-1381.0]	**<0.001**
HSTNT, PG/ML	187 / 89 / 98	27.0 [13.0-54.0]	52.0 [30.5-80.5]	14.0 [8.8-25.3]	**<0.001**

In the total cohort, the median age is around 72 years, but there is significant age difference in both disease groups, as Amyloidosis patients are much older. In total, 31.6% are female, whereby the distribution is unequal, showing more women in the HCM collective. One can see that HCM patients in this cohort have a higher blood pressure than CA patients, on average 140/84 compared to 134/78 mmHg. Patients with HCM show also higher BMI values. Over half of HCM patients have NYHA class I or II, on the other side CA patients have mostly NYHA class II or III. As systemic

amyloidosis can affect the kidneys as well, and the disease cohort is older, the protein creatinine ratio and the estimated glomerular filtration rate are significantly worse than in HCM patients.

3.2 Echocardiographic indices – Total cohort

TABLE 2: ECHOCARDIOGRAPHIC INDICES

	Available in N (total/CA/HCM) (%)	Total cohort N = 195	CA N = 95	HCM N = 100	P-value
STRUCTURAL PARAMETERS					
AORTA DIAMETER, CM	158 / 71 / 87 (81.0)	3.3 ± 0.5	3.4 ± 0.4	3.2 ± 0.5	**0.002**
IVS END DIASTOLIC DIAMETER, CM	164 / 79 / 85 (84.1)	1.9 ± 0.4	1.8 ± 0.4	1.9 [1.7-2.2]	0,351
LV END DIASTOLIC DIAMETER, CM	165 / 79 / 86 (84.6)	4.2 [3.9-4.7]	4.3 ± 0.7	4.2 [3.9-4.6]	0.907
LV POSTERIOR WALL END DIASTOLIC DIAMETER, CM	164 / 79 / 85 (84.1)	1.4 [1.2-1.7]	1.6 ± 0.4	1.3 ± 0.3	**<0.001**
LV MASS, G	160 / 77 / 83 (82.1)	298.0 [235.9-371.5]	327.8 ± 104.5	269.8 [230.3-349.2]	**0.019**

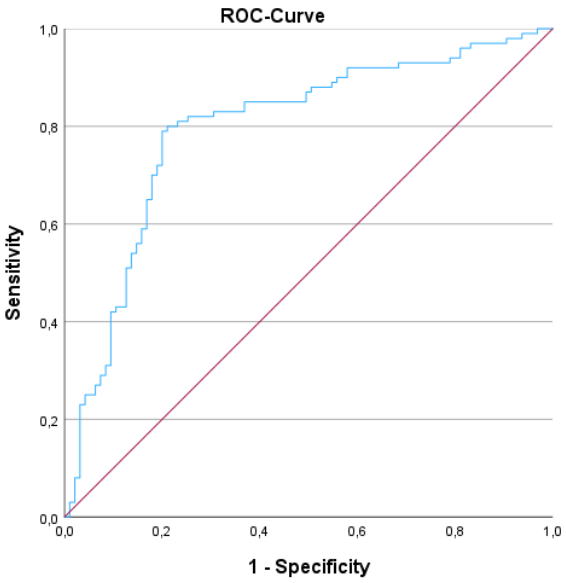
	AVAILABLE IN N (TOTAL/CA/HCM) (%)	TOTAL COHORT N = 195	CA N = 95	HCM N= 100	P-VALUE
DIASTOLIC DYSFUNCTION					
E/A RATIO	131 / 48 / 83 (67.2)	1.2 [0.8-1.7]	1.3 [1.0-2.1]	1.1 [0.8-1.4]	**0,008**
TR SYSTOLIC JET VELOCITY, M/S	129 / 60 / 69 (66.2)	2.6 ± 0.4	2.6 [2.4-2.7]	2.5 ± 0.4	0,118
AVERAGE E/E' RATIO	169 / 80 / 89 (86.7)	12.7 [9.6-15.9]	13.9 [11.8-17.6]	11.1 [8.6-13.8]	**<0.001**
LA VOLUME INDEX, ML/M²	125 / 62 / 63 (64.1)	52.5 [44.3-65.8]	54.3 [48.5-63.1]	51.4 [39.2-66.7]	0.366
MITRAL INFLOW E MAXIMAL VELOCITY, M/S	178 / 84 / 94 (91.3)	0.8 [0.6-0.9]	0.8 [0.7-0.9]	0.8 [0.6-0.9]	0.278

DT OF E, MS	158 / 71 / 87 (81.0)	207.5 [171.0-255.3]	191 [156-232]	231.8 ± 73.1	**0.006**
MITRAL INFLOW A MAXIMAL VELOCITY, M/S	134 / 49 / 85 (68.7)	0.6 [0.5-0.9]	0.6 ± 0.3	0.7 [0.5-0.9]	**0.003**
E' SEPTAL, M/S	173 / 82 / 91 (88.7)	0.06 [0.05-0.07]	0.05 [0.04-0.06]	0.06 [0.05-0.08]	**<0.001**
E' LATERAL, M/S	172 / 81 / 91 (88.2)	0.07[0.06-0.09]	0.06 [0.05-0.08]	0.08[0.07-0.1]	**<0.001**
RUPV S WAVE, M/S	120 / 59 / 61 (61.5)	0.4 [0.3-0.5]	0.3[0.2-0.4]	0.5 ± 0.1	**<0.001**
RUPV D WAVE, M/S	119 / 58 / 61 (61.0)	0.5 [0.4-0.7]	0.6 ± 0.2	0.4 [0.3-0.5]	**<0.001**
S/D RATIO	119 / 58 / 61 (61.0)	0.8 [0.5-1.3]	0.5 [0.4-0.9]	1.2 ± 0.4	**<0.001**

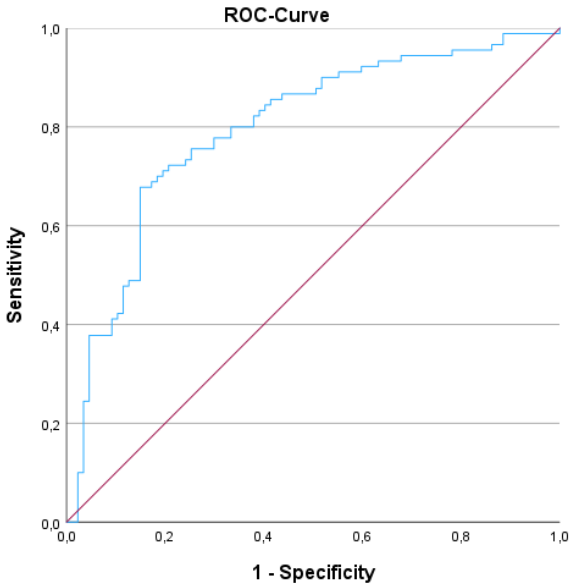
Looking at structural parameters on echocardiography, there are significant differences between both cohorts. CA patients have higher values in aorta diameter, LV posterior wall end diastolic diameter and LV mass. Only interventricular septum diameter and LV end diastolic diameter are similar in both cohorts.

The parameters of diastolic dysfunction vary significantly between both cohorts, particularly E/A ratio, E/e' ratio, deceleration time of E, A maximal velocity, e' septal and medial, S & D wave as well as S/D ratio. Interestingly, LA volume index is similar in both, and in total cohort measures around 52.5 ml/m². TR systolic jet velocity and E maximal velocity are remarkably similar in both cohorts.

3.3 Diagnostic accuracy



LA Strain ROC Curve



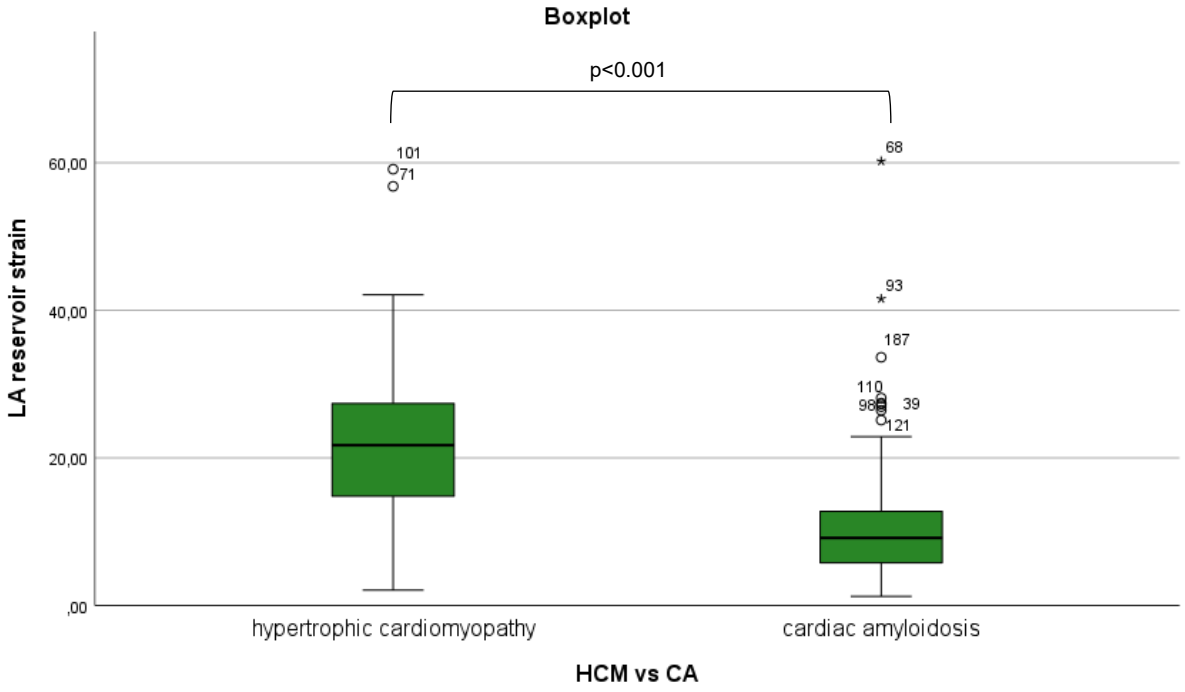
RA strain ROC curve

	LA STRAIN	RA STRAIN
AREA UNDER CURVE (CONFIDENCE INTERVAL)	0.796 [0.731; 0.861]	0.795 [0.728; 0.863]
CUT OFF VALUE (%)	14.3	23.7
SENSITIVITY (%)	79.0	67.8
SPECIFICITY (%)	80.0	85.1
YOUDEN-INDEX	0.59	0.53

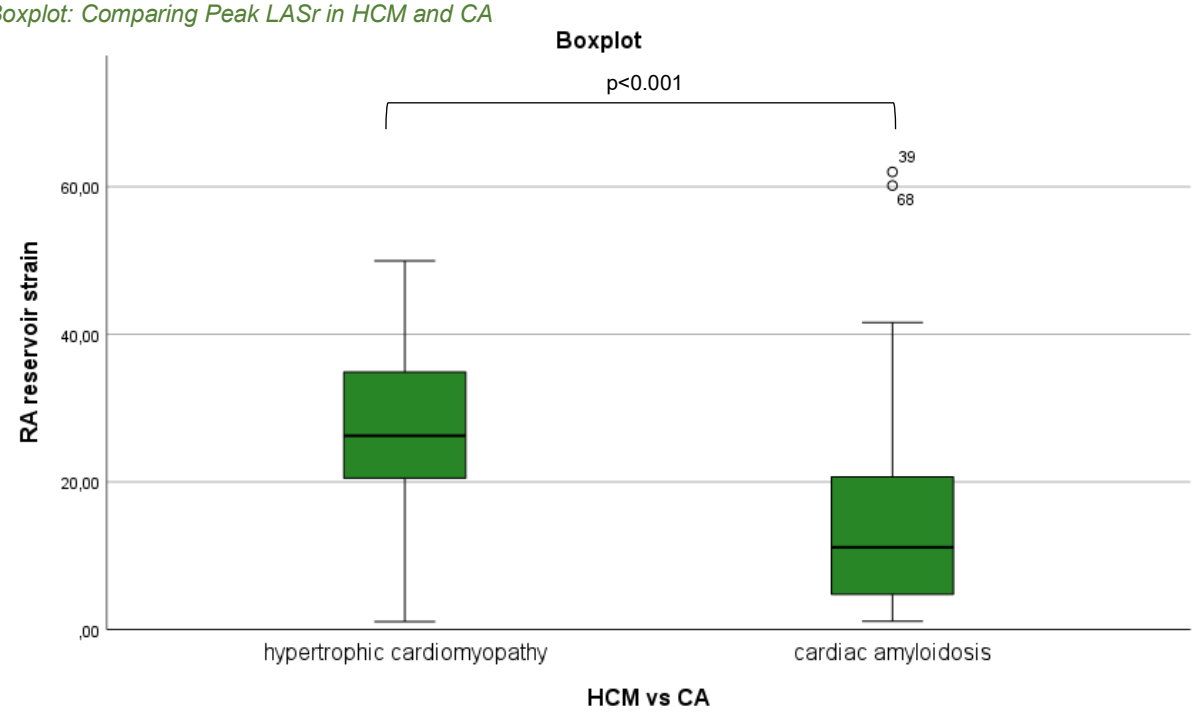
For the diagnostic accuracy, a ROC analysis was performed. Both graphs show the ROC curve as well as the reference line. By using the coordinates of the ROC curve and by calculating the Youden Index a cut off value can be determined.

The area under the curve for LA strain is 0.796 [CI: 0.731; 0.861], similarly for RA strain 0.795 [CI: 0.728; 0.863]. LA strain values above 14.3 % implicate a diagnosis of HCM with a sensitivity of 79% and specificity of 80%. A cut off value of 23.7% in RA strain implicates HCM with a sensitivity of 68% and specificity of 85%.

3.4 Comparison of atrial strain – total cohort



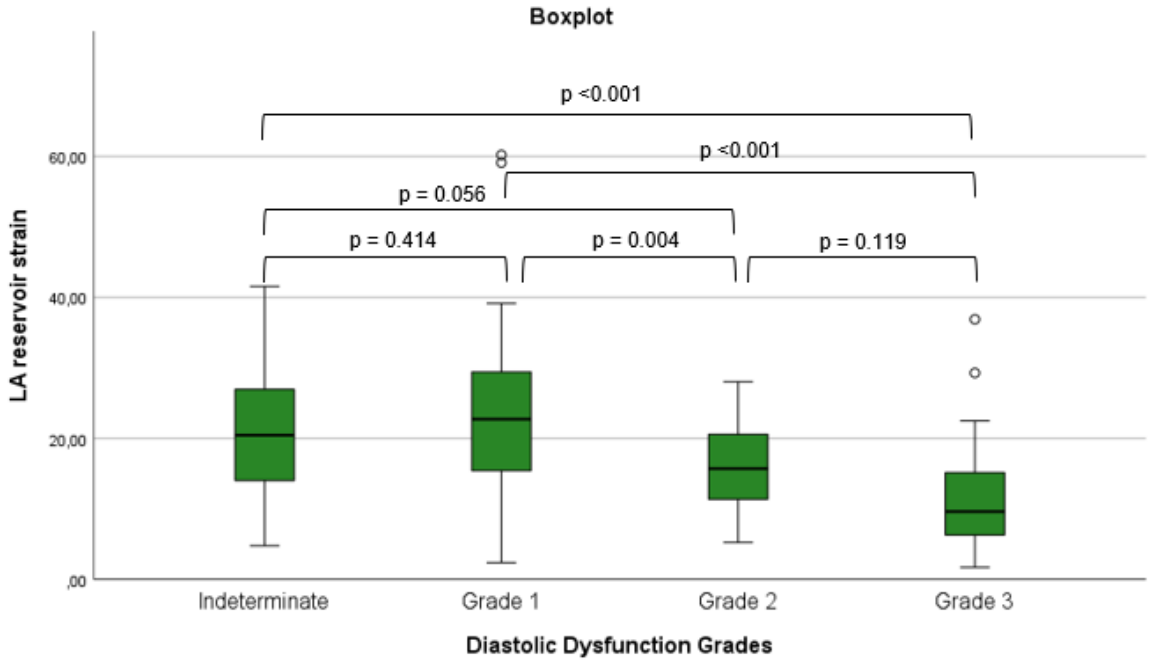
Boxplot: Comparing Peak LASr in HCM and CA



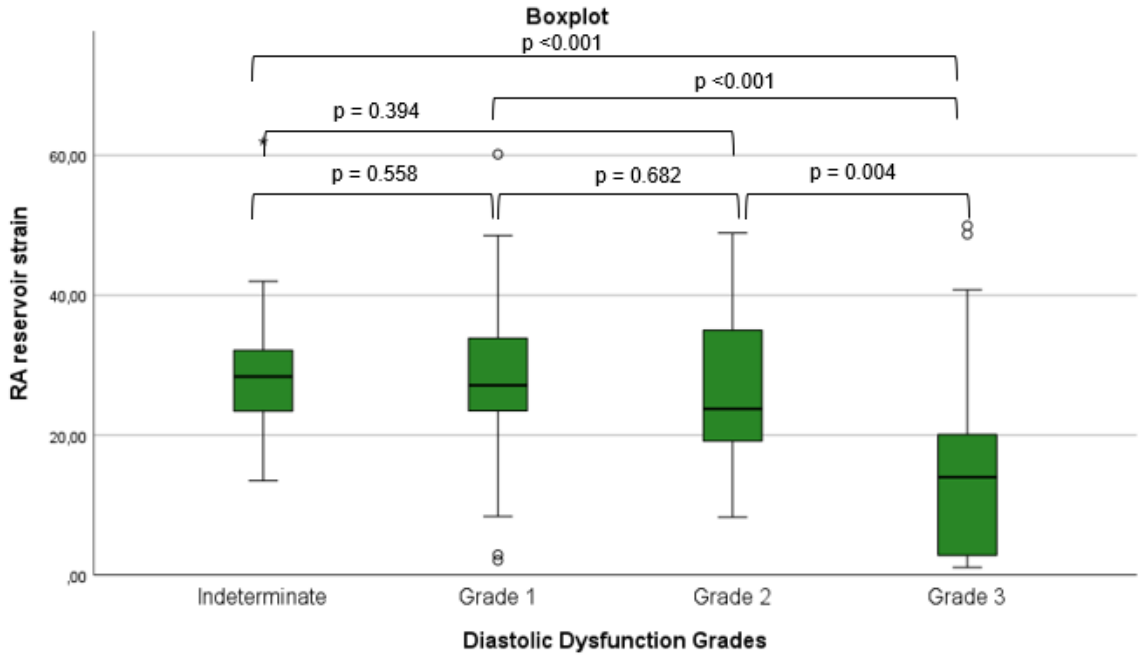
Boxplot: Comparing Peak RASr in HCM and CA

Generally, both atrial strains differ significantly between both diseases, with P-values under 0.001. The median left atrial strain is 21.7% (14.8-27.6) in HCM collective and 9.2% (5.6-13.0) in CA patients. The average RA strain in HCM collective is 26.7±11.9% and median RA strain is 11.1% (4.5-20.9) in CA patients.

3.5 Atrial strain stratified according to diastolic dysfunction grades



Boxplot: Comparing LA strain in Diastolic Function Grades



Boxplot: Comparing RA strain in Diastolic Function Grades

When looking at atrial strain stratified according to diastolic dysfunction, one can see a clear decrease in atrial function with worsening diastolic function. Particularly, there are significant differences between indeterminate and Grade 3 as well as Grade 1 and 3 in both LA and RA strain. There is also a significant difference between Grade

1 and 2 in LA strain, whereas this significant difference can be found between Grade 2 and 3 in RA strain.

3.6 Baseline characteristics – matched cohort

TABLE 4: BASELINE CHARACTERISTICS.

	Available in N (total/CA/HCM) (%)	Total Cohort N=74	CA N=37	HCM N=37	P-Value
BASELINE CHARACTERISTICS					
AGE, YEARS	74 (100)	70.5 [16.5]	67.8 ± 12.0	72 [17]	0,909
FEMALE, N (%)	74 (100)	42 (56.8)	21 (56.8)	21 (56.8)	1
SBP, MMHG	64 / 30 / 34 (86.5)	137.5 [32]	131.4 ± 18.4	149 [32.3]	0.005
DBP, MMHG	64 / 30 / 34 (86.5)	83.7 ± 12.1	81.2 ± 11.3	86.0 ± 12.4	0.111
BMI, KG/M²	70 / 33 / 37 (94.6)	25.3 [5.4]	24.4 [5.3]	26.9±4.0	0.125
NYHA CLASS I, N (%)		20 (29)	8 (25)	12 (32.4)	
NYHA CLASS II, N (%)		22 (31.9)	10 (31.3)	12 (32.4)	
NYHA CLASS II-III, N (%)		9 (13)	5 (15.6)	4 (10.8)	
NYHA CLASS III, N (%)		16 (23.2)	9 (28.1)	7 (18.9)	
NYHA CLASS IV, N (%)		2 (2.9)	0 (0)	2 (5.4)	
LABORATORY INDICES					
PROTEIN CREATININE RATIO, MG/G	64 / 31 / 33 (86.5)	125 [119.5]	129 [119]	106 [112.5]	0.088
EGFR, ML/MIN/1.73M²	72 / 35 / 37 (97.3)	66.5 ± 22.2	64 ± 22.7	69 ± 21.7	0.34

In the age- and sex-matched cohort, there are 56.8% female with a median age of around 71 years. There are no significant differences in baseline characteristics, expect systolic blood pressure, which is still higher in HCM patients in this cohort. The distribution of NYHA class is more evenly distributed, but CA patients still show higher NYHA classes.

3.7 Echocardiographic indices – matched cohort

TABLE 5: ECHOCARDIOGRAPHIC INDICES

	Available in N (total/CA/HCM) (%)	Total cohort N = 74	CA N = 37	HCM N = 37	P-value
STRUCTURAL PARAMETERS					
AORTA DIAMETER, CM	57 / 24 / 33 (77.0)	3.2 ± 0.4	3.2 ± 0.4	3.3 ± 0.5	0.877

IVS END DIASTOLIC DIAMETER, CM	60 / 29 / 31 (81.1)	1.9 ± 0.4	1.7 ± 0.4	2.0 ± 0.4	**0.013**
LV END DIASTOLIC DIAMETER, CM	61 / 29 / 32 (82.4)	4.1 ± 0.7	4.1 ± 0.7	4.1 ± 0.7	0.97
LV POSTERIOR WALL END DIASTOLIC DIAMETER, CM	60 /29 / 31 (81.1)	1.4 ± 0.3	1.5 ± 0.4	1.4 ± 0.3	0.053
LV MASS, G	59 / 29 / 30 (79.7)	293.3 ± 96.8	286.1 ± 100.0	300.2 ± 94.7	0.582

	AVAILABLE IN N TOTAL/CA/HCM) (%)	**TOTAL COHORT N = 74**	**CA N = 37**	**HCM N= 37**	**P-VALUE**
DIASTOLIC DYSFUNCTION					
E/A RATIO	54 / 22 / 32 (73)	1.2 [0.7]	1.6 ± 0.7	0.9 [0.6]	**0.017**
TR SYSTOLIC JET VELOCITY, M/S	47 / 17 / 30 (63.5)	2.6 ± 0.4	2.6 ± 0.3	2.6 ± 0.4	0.721
AVERAGE E/E' RATIO	64 / 29 / 35 (86.5)	12.7 [7.0]	13.5 ± 4.4	11.5 [6.5]	0.312
LA VOLUME INDEX, ML/M²	48 / 22 / 26 (64.9)	53.3 ± 17.2	49.7 ± 13.5	56.3 ± 19.6	0.187
MITRAL INFLOW E MAXIMAL VELOCITY, M/S	67 / 31 / 36 (90.5)	0.8 ± 0.2	0.8 ± 0.2	0.8 ± 0.2	0.892
DT OF E, MS	62 / 29 / 33 (83.8)	225.5 ± 72.0	196 [84.5]	237.5 ± 72.6	0.122
MITRAL INFLOW A MAXIMAL VELOCITY, M/S	54 / 22 / 32 (73)	0.7[0.5]	0.6 ± 0.2	0.9 ±0.4	**<0.001**
E' SEPTAL, M/S	65 / 30 / 35 (87.8)	0.06 [0.02]	0.06 [0.02]	0.06 ± 0.02	0.707
E' LATERAL, M/S	64 / 29 / 35 (86.5)	0.07 [0.03]	0.06 [0.03]	0.08 ± 0.03	0.161
RUPV S WAVE, M/S	45 / 22 / 23 (60.8)	0.4 ± 0.2	0.4 ± 0.2	0.5 ± 0.2	**0.011**
RUPV D WAVE, M/S	45 /22 / 23 (60.8)	0.5 ± 0.2	0.5 ± 0.2	0.5 ± 0.2	0.376
S/D RATIO	45 / 22 / 23 (60.8)	0.9 [0.8]	0.8 [0.8]	1.1 ± 0.5	**0.022**

Even echocardiographic structural parameters are similar in both cohorts, expect interventricular septum diameter, which shows higher values in HCM patients. Diastolic function parameters are as well very similar in both groups, only showing significant differences in E/A ratio, A maximal velocity, S wave and S/D ratio.

3.8 correlation of cardiac parameters and atrial strain – matched cohort

Correlation cardiac parameters and atrial strain – cardiac amyloidosis

SPEARMAN-RHO-COEFFICIENT (P-VALUE)	LA STRAIN	RA STRAIN
NTPROBNP	-0.5 (0.001)	-0.4 (0.011)
TROPONIN T	-0.7 (<0.001)	-0.6(<0.001)

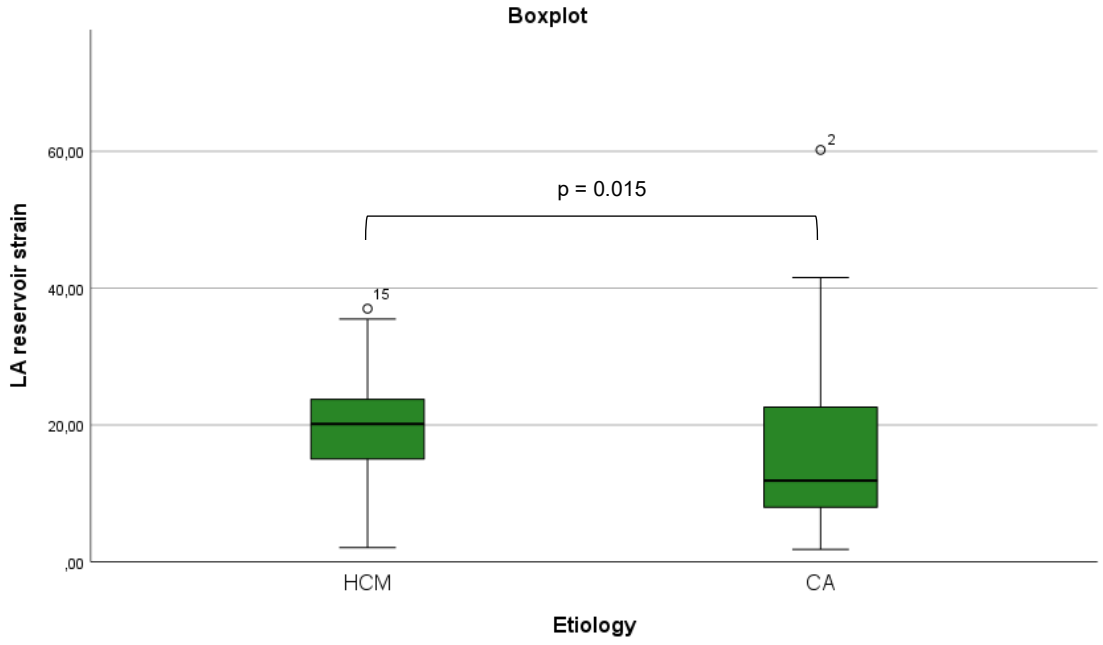
Correlation cardiac parameters and atrial strain – hypertrophic cardiomyopathy

SPEARMAN-RHO-COEFFICIENT (P-VALUE)	LA STRAIN	RA STRAIN
NTPROBNP	-0.5 (<0.001)	-0.6 (<0.001)
TROPONIN T	-0.3 (0.077)	-0.4 (0.033)

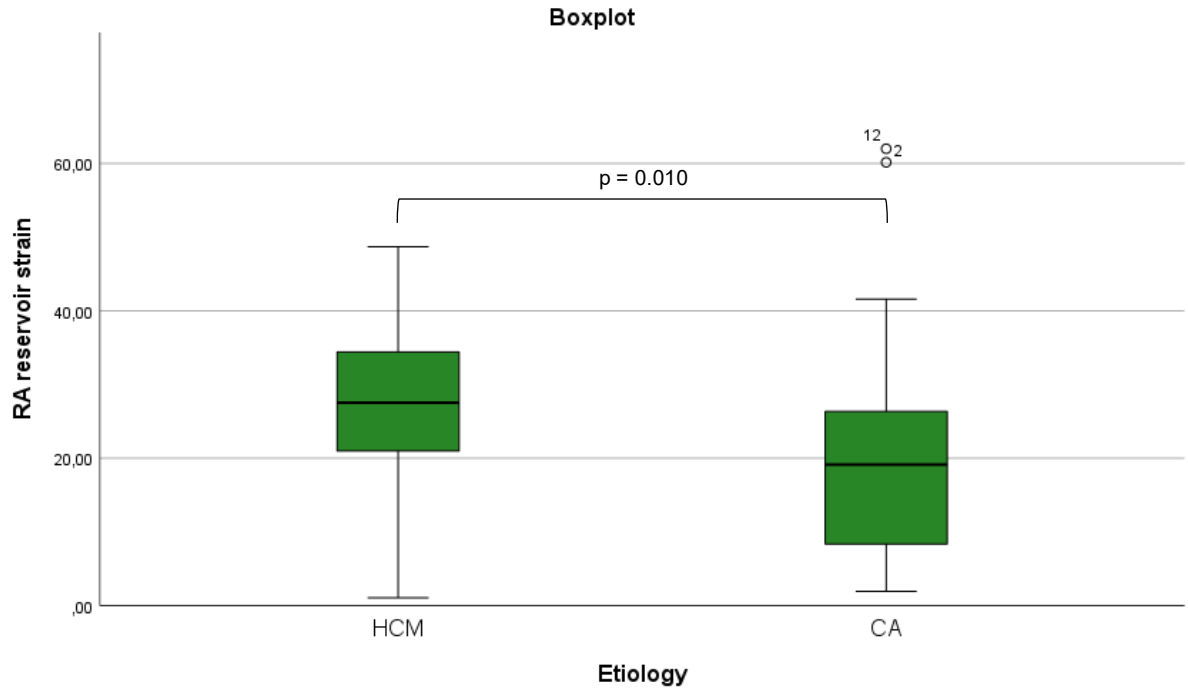
In CA patients, NT-proBNP and Troponin T have a significant negative correlation with atrial strain. Particularly Troponin T has strong correlations with LA strain (-0.7, $p < 0.001$).

In HCM patients, a similar pattern is present, expect there is no significant correlation between Troponin T and LA strain. But a strong negative correlation between NT-proBNP and RA strain (-0.6, $p<0.001$) can be found.

3.9 comparison of atrial function – matched cohort



Boxplot: Comparing Peak LASr in HCM and CA



Boxplot: Comparing Peak RASr in HCM and CA

Even when matched and presenting very similar characteristics in echocardiography, LA and RA strain differ significantly in both cohorts, showing higher values in HCM patients. The average left atrial strain is 19.6±8.3% in HCM collective and median LA strain is 11.9% (15.1) in CA patients. The average RA strain in HCM collective is 26.0±12.4% and median RA strain is 19.1% (18.5) in CA patients.

4 Discussion

4.1 Short summary of main results

The main finding of this study is that atrial reservoir strain differs significantly between CA and HCM (LASr 9.2% vs. 21.7%, $p<0.001$; RASr 11.1% vs. 26.7%, $p<0.001$). Atrial reservoir strain is also associated with the severity of diastolic dysfunction. Through ROC analysis, a optimal cut-off value of ≤ 14.3% for LASr and 23.7% for RASr was determined. Lower values implicate CA. Area under the curve for LASr and RASr was 0.8 with 79% sensitivity and 80% specificity for LASr and 68% sensitivity and 85% specificity for RASr.
A sensitivity analysis in the age- and sex-matched cohort showed unchanged results. Evidently, even after adjusting for the most important confounders, age and sex, LASr and RASr still differ significantly between both disease cohorts.

4.2 Atrial Strain differs between CA and HCM

As one can see, the left and right atrial strain decreases in both disease cohorts as the grade of diastolic dysfunction increases. This can be explained by the heightened afterload the atria face due to ventricular stiffness and/or increased ventricular pressure. Additionally, the descent of the atrioventricular plane decreases when the myocardial stiffness prevents proper contraction. This leads to decreasing atrial reservoir function, which in return is part of the diastolic function. In case of cardiac amyloidosis, amyloid infiltration of atrial myocardium is possible, therefore the wall stiffness increases, causing loss of distensibility, which prevents atrial enlargement.(29-33)
As a result of Mann-Whitney-U-Test, we found a significant difference in both atrial strains between both diseases. The question arises, why is that?
As Nochioka et al. stated in his paper, atrial pathology in Cardiac amyloidosis has a complex pathophysiology. It can be hypothesized that; it is a combination of heightened filling pressures and the typical restrictive pattern of the left ventricle due to intramyocardial amyloid infiltration as well as a result of direct amyloid infiltration in the atria an intrinsic LA failure. Kwong et al has shown in his study of LGE in CA patients a high prevalence of LGE in LA walls of cardiac amyloidosis.

HCM patients suffer from a disorganisation of hypertrophic myocytes and interstitial fibrosis, which results in a stiff ventricle and rising LV pressures.(8) But HCM typically does not affect LA function primarily. LA function just worsens because of the heightened afterload and distension. Therefore, the added load of intrinsic LA failure could explain the lower values of LA strain in CA patients.
Little is known about the right atrium, and its status in pathophysiology of CA and HCM. Interestingly the cut-off value found in this study, is the same as Hosseinsabet et al. stated in his study for normal ranges of RA strain. This could implicate, that HCM patients have a normal RA function, as the right heart side is not typically affected in these patients. On the contrary, patients with CA can show amyloid infiltration of RV as well as RA, which explains the lower RA strain values. (34)

Even after adjustment for the most important confounders, age and sex, there is still a significant difference in atrial function. In females, the atrial strain values are typically higher and in the total cohort, the sex is unequally distributed. This could have resulted in false results. The same applies for age. CA patients are typically older than HCM patients, this could as well have influenced the atrial strain. But after adjustment, most characteristics are similar in both groups, and atrial strain still differs significantly, implicating a difference in atrial function between both cardiomyopathies. The possible explanation was discussed earlier and applies here as well.(35)

4.3 Atrial strain is associated with disease severity in HCM and CA

In Cardiac Amyloidosis, LA and RA strain have a significant strong negative correlation with Troponin T as well as NTproBNP. Troponin T can be elevated because of oxidative stress and consequently cell death induced by amyloidogenic protein. NTproBNP is commonly used as a marker of heart failure. The level increases when ventricular pressure increases, causing the myocardium to overly stretch and the wall tension to increase. Furthermore, Damy et al. found a significant correlation of NTproBNP and severity of amyloid deposition. (36) Most importantly, both cardiac parameters are subject to renal clearance and Amyloidosis patients often have renal involvement. Therefore, the elevated markers are probably a combination of a lack of clearance and increased production. (37, 38)

In HCM, LA and RA strain correlate significantly with NTproBNP. Many studies showed an association of NTproBNP and markers of diastolic function in hypertrophic cardiomyopathy patients.(38-40) As LASr is also subject to diastolic function, and association with NTproBNP seems plausible. Troponin T has only a weak, negative correlation with atrial strain. There are many theories that explain Troponin T elevations in HCM patients. It can be hypothesized that due to heightened oxygen demand and reduced capillary density caused by the hypertrophic myocardium, myocardial ischemia appears. It is also discussed, that through increased pressure the subendocardial perfusion is limited.(38, 39)
As NTproBNP and Troponin T are typically used to stage cardiac diseases, LASr and RASr could be used to measure disease severity and be part of new staging systems.

4.4 Diagnostic accuracy of LASr and RASr

LASr and RASr show high diagnostic accuracy in differentiating between HCM and CA. Our data is supported by other studies, which show similar results. Monte et al. compared ATTR-CA and HCM and the average level of LASr is strikingly similar (9% in ATTR-CA and 14.5% in HCM). Also Mattig et al. reported an average RASr value of 12 ± 8% in Cardiac Amyloidosis patients. Furthermore, Brand et al. found average LASr values of 9.7±5.2% in AL and ATTR CA, and they found that LASr has a higher diagnostic accuracy then RELAPS when compared to patients with unknown left ventricular hypertrophy. They calculated a cut-off value of < 15.8%, which is similar to our results. In this study, much more patients were included then in the studies listed above, but still showing the same results.(41-43)
The question arises, why a new echocardiographic variable is needed to quantify the left atrium, when LA volume index is already part of the diagnostic algorithm. But LAVi solely quantifies LA volume indexed by BSA, but there are cases, specifically in CA, where atrial distension is not even possible, due to amyloid infiltration. Moreover, atrial function plays an important role in diastolic function, as it contributes to systolic filling of the ventricles. Which is something that LAVi simply cannot quantify. LAVi only represents atrial distension as a result of heightened atrial afterload, which in turn is due to increased ventricular pressure.(29, 31-33, 44)

4.5 Summary of main findings

This study took a closer look at atrial function in HCM and CA patients. 196 patients were included, respectively 100 patients with HCM and 96 patients with CA, between both cohorts was a significant difference in left and right atrial function. This study also provides a cut off value for atrial strain to assist the diagnostic process and differentiation of HCM and CA. Even after adjustment for confounder, by matching for sex and age, we still could show a significant difference between both disease entities.

4.6 Perspectives

This data is a steppingstone for further investigation of LA myopathy in HCM and CA and its possible implications for diagnosis and disease monitoring. A prospective validation is needed to generate new algorithms for assessing grade of diastolic dysfunction, as LASr is probably superior to previous parameters like LAVi. Furthermore, atrial strain could prove itself to be an indicator of disease progression, due to its association with markers of disease severity (NTproBNP and hs-TnT).

4.7 Limitations

There are some limitations to report. This is a single-center study, therefore generalizability of these results in limited. The patients were recruited in a tertiary centre, which increases the probability of including patients with later disease stages. Furthermore, only a small number of patients was included, therefore studies with larger study populations are needed to validate the data. There were no assumptions made on causality.

4.8 Conclusion

To conclude this thesis, we took a closer look at atrial reservoir function in two disease entities, CA and HCM. We showed a significant difference in atrial reservoir function in left and right atrium between both cohorts. Both patient groups have impaired left atrial reservoir function, on the right side only CA patients show reduced

atrial reservoir function. Furthermore, LASr and RASr could prove itself, as a monitor for disease severity, as they are associated with NTproBNP and Troponin T levels. Atrial reservoir function could also be used as a parameter for diastolic dysfunction, as it is associated with the severity of diastolic dysfunction. Most importantly, we demonstrated a high diagnostic accuracy of LASr and RASr in differentiating both diseases. A cut-off value of 14.3% and 23.7%, respectively for LASr and RASr, has a high sensitivity and specificity. Even after adjusting for the most important confounders, age and gender, we still demonstrated a significant difference in atrial reservoir function.

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